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Ezetimibe and Simvastatin Reduce Inflammation, Disease Activity, and Aortic Stiffness and Improve Endothelial Function in Rheumatoid Arthritis

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Objectives The aim of this study was to investigate the effect of simvastatin and ezetimibe on inflammation, disease activity, endothelial dysfunction, and arterial stiffness in a cohort of rheumatoid arthritis (RA) patients.

Background Rheumatoid arthritis is a chronic inflammatory condition associated with increased cardiovascular risk. Statins reduce inflammation and disease activity in RA patients, but whether this is due to pleiotropism or cholesterol lowering per se is unclear.

Methods Twenty patients received 20 mg simvastatin or 10 mg ezetimibe each for 6 weeks in a randomized double-blind crossover study. Disease activity, blood pressure, aortic pulse wave velocity (PWV), brachial artery flow-mediated dilation (FMD), and serum inflammatory markers were measured before and after each treatment.

Results Both ezetimibe and simvastatin significantly reduced total cholesterol (-0.62 ± 0.55 mmol/l and -1.28 ± 0.49 mmol/l, respectively; $p < 0.001$), low-density lipoprotein cholesterol (-0.55 ± 0.55 mmol/l and -1.28 ± 0.49 mmol/l; $p < 0.0001$), and C-reactive protein (-5.35 ± 9.25 mg/l and -5.05 ± 6.30 mg/l; $p < 0.001$). Concomitantly, Disease Activity Score (-0.55 ± 1.01 and -0.67 ± 0.91; $p = 0.002$), aortic PWV (-0.69 ± 1.15 m/s and -0.71 ± 0.71 m/s; $p = 0.001$), and FMD ($1.37 \pm 1.17\%$ and $2.51 \pm 2.13\%$; $p = 0.001$) were significantly improved by both drugs.

Conclusions This study demonstrates that both ezetimibe and simvastatin reduce disease activity and inflammatory markers to a similar extent in patients with RA. Therapy is also associated with a concomitant reduction in aortic PWV and improvement in endothelial function. This suggests that cholesterol lowering per se has anti-inflammatory effects and improves vascular function in RA. (J Am Coll Cardiol 2007;50:852–8) © 2007 by the American College of Cardiology Foundation

Rheumatoid arthritis (RA) is associated with increased mortality and comorbidity, mostly owing to an excess of cardiovascular disease (1). This cannot solely be explained by traditional cardiovascular risk factors (2), which has led to the suggestion that the chronic systemic inflammation characterizing RA may accelerate the atherosclerotic process either directly by contributing to plaque formation and destabilization or indirectly via endothelial dysfunction and aortic stiffening. Aortic stiffening increases wave reflection, thus increases pulse pressure, leading to elevated left ventricular (LV) load and a possible LV hypertrophy, thus increased cardiovascular risk. Both aortic stiffening and endothelial dysfunction predict future cardiovascular risk in other patient groups (3,4) and may play a pathophysiologic role in atheroma formation. Moreover, we and others have demonstrated both endothelial dysfunction (5,6) and increased arterial stiffness (7–9) in RA, which can be reversed by successful anti-inflammatory therapy (9,10).

Statins (3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors) are effective in reducing cardiovascular morbidity and mortality in a variety of populations (11,12). In addition to cholesterol reduction, a number of other pleiotropic effects have been described with statins that may improve outcome independently of cholesterol reduction (13). One such effect is a reduction in inflamma-

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tion (14), which is increasingly thought to play an important role in the pathogenesis of atherosclerosis. The TARA (Trial of Atorvastatin in Rheumatoid Arthritis) study assessed the effect of 6 months' treatment with atorvastatin in patients with RA. Statin therapy was associated with a marked reduction in inflammatory markers (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]) as well as with a reduction of disease activity (15). That study did not include any control cholesterol-lowering therapy and it is therefore unclear whether the observed anti-inflammatory properties were a pleiotropic effect of statins or simply due to a reduction in cholesterol. Moreover, the authors did not assess the effect of therapy on vascular function.

The aim of the present study was to test the hypothesis that cholesterol reduction per se reduces inflammation, disease activity, and surrogate measures of cardiovascular risk in patients with RA, by examining the effect of simvastatin and a nonstatin, ezetimibe, which acts locally by inhibiting cholesterol absorption from the small intestine, in a randomized double-blind crossover study.

Methods

Study population. Twenty patients with active RA who met the 1987 American Rheumatism Association criteria were recruited from the rheumatology clinics at Addenbrooke's Hospital, Cambridge, United Kingdom. Inclusion criteria included a Disease Activity Score (DAS)-28 >3.5 and a serum CRP >6 mg/l. Individuals with cardiovascular disease, untreated hypertension (blood pressure ≥140/90 mm Hg), diabetes, hypercholesterolemia (total cholesterol ≥6.5 mmol/l), renal disease, and current smokers were excluded, because these conditions are associated with endothelial dysfunction and arterial stiffening. Patients on vasoactive drugs were also excluded. We also randomly selected 20 age- and gender-matched control subjects from our database to compare the baseline arterial stiffness and endothelial function between patients with RA and healthy individuals. Approval was obtained from the Local Research Ethics Committee, and written informed consent was obtained from each participant.

Hemodynamic measurements. All studies were conducted in a quiet temperature-controlled room. Blood pressure was recorded in the brachial artery using a validated oscillometric technique (HEM-705CP, Omron Corp., Kyoto, Japan). Radial artery waveforms were obtained with a high-fidelity micromanometer (SPC-301, Millar Instruments, Houston, Texas) from the wrist, and a corresponding central waveform was generated using a validated transfer function (Sphygmocor, AtCor Medical, Sydney, Australia). Augmentation index (AIx), a composite measure of systemic arterial stiffness and wave reflection, mean arterial pressure, and heart rate was determined using the integrated software. Aortic (carotid to femoral) pulse wave velocity (PWV) was measured as previously described (16).

Abbreviations and Acronyms

AIx = augmentation index
CRP = C-reactive protein
DAS = Disease Activity Score
ESR = erythrocyte sedimentation rate
FMD = flow-mediated dilatation
GTN = glyceryl trinitrate
oxLDL = oxidized low-density lipoprotein
PWV = pulse wave velocity
RA = rheumatoid arthritis

Endothelial function was assessed in the brachial artery using the technique of flow-mediated dilatation (FMD) (17). Vessel diameter was measured using high-resolution vascular ultrasound (Acuson 128XP/10, Siemens, Erlangen, Germany) with a 10-MHz linear array transducer. Brachial artery diameter was measured continuously for 1 min at baseline and for a further 5 min after cuff deflation. The cuff was placed below (distal to) the ultrasound transducer and inflated to 200 mm Hg for 5 min. After return to baseline, vessel diameter was again measured continuously for 5 min after administration of 25 μg sublingual glyceryl trinitrate (GTN). The FMD was defined as the maximum percentage increase in vessel diameter during reactive hyperemia; GTN-mediated dilatation was defined as the maximum percentage increase in vessel diameter after sublingual GTN. The FMD recordings were analyzed off line by a blinded operator unfamiliar with the study.

Laboratory measurements. Fasting lipid profile, blood glucose, and high-sensitivity CRP, ESR, and rheumatoid factor were determined using standard methodology. Oxidized low-density lipoprotein (oxLDL) was measured in stored serum samples (−80°C) by a commercially available solid-phase two-site enzyme immunoassay (Mercodia, Uppsala, Sweden). The samples were measured in a single analytical run.

Disease Activity Score. The DAS-28 is a validated composite Disease Activity Score (18). The components of DAS-28 include the number of swollen and tender joints from 28 assessed joints, ESR, and patient-assessed visual analog score of overall well-being (scaled 0 to 100). The DAS-28 was calculated as previously described (18).

Experimental protocol. The present study was conducted in a randomized double-blind crossover manner. Following a 2-week placebo run-in, patients received 10 mg ezetimibe or 20 mg simvastatin, in random order, each for 6 weeks, with a 6-week washout between drugs (Fig. 1). All hemodynamic measurements were assessed at baseline, following the end of the placebo run-in, and at the end of each 6-week period (drug 1, washout, and drug 2). Blood was drawn at each time point for the measurement of biochemical markers, and DAS-28 was calculated.

Data analysis. Data were analyzed using SPSS software (version 12, SPSS Inc., Chicago, Illinois). Two-way repeated measures analysis of variance was used to investigate the effect of the drugs. Custom hypothesis testing (simple) of within-subject contrasts was performed for the ezetimibe-simvastatin comparison, where treatment order was entered as a between-subject factor. In post hoc tests,

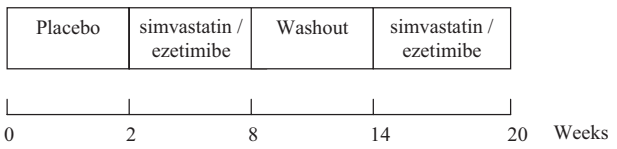

Figure 1 The Study Design

Following 2-week placebo run-in, patients received simvastatin or ezetimibe in a randomized double-blinded manner, each for 6 weeks, with a 6-week washout between the drugs.

the effect of individual treatments was determined using paired Student *t* tests with Bonferroni adjustment for 2 comparisons. For the skewed variables (CRP and ESR) log-transformed values were used for the analyses. The carryover effect of the drugs was assessed with paired Student *t* tests between the baseline measurements at week 2 (baseline 2) and week 14 (end of washout period). Pearson correlations were calculated between absolute changes in lipid parameters and anti-inflammatory markers, hemodynamic measures and disease activity. A probability of <0.05 was considered to be significant. Data are given as mean ± SD.

Results

A total of 20 patients with active rheumatoid arthritis completed the study. The demographic variables and biochemical and hemodynamic characteristics of patients at entry are shown in Table 1. There was no difference in the lipid profile between patients with RA and control subjects (total cholesterol 5.3 ± 0.9 mmol/l vs. 4.9 ± 1.3 mmol/l; LDL 3.1 ± 0.9 mmol/l vs. 2.8 ± 0.9 mmol/l, respectively; both $p = 0.3$). The current therapy included methotrexate ($n = 13$), other disease-modifying drug ($n = 7$), nonsteroidal anti-inflammatory drug ($n = 14$), and prednisolone ($n = 9$) (mean dose 6.7 ± 3.2 mg). Most patients were taking 2 or more drugs concomitantly ($n = 18$), and none were free from medication. None of the patients were receiving medication for hypertension or hypercholesterolemia. Patients remained on their existing therapy, and they did not receive corticosteroid injections throughout the study period.

Table 2 shows the effect of ezetimibe and simvastatin on lipids, inflammatory markers, disease activity, and hemodynamic parameters after 6 weeks of each drug therapy; the presented p values in the "Between Drugs" column are Bonferroni adjusted. Both ezetimibe and simvastatin produced a significant reduction in total cholesterol, LDL cholesterol, and oxLDL. The reduction was more pronounced with simvastatin than with ezetimibe. Baseline oxLDL was significantly higher in patients with RA compared with control subjects (62.8 ± 16.6 U/l vs. 43.6 ± 12.9 U/l, respectively; $p < 0.001$). The ESR and CRP were reduced by both ezetimibe and simvastatin. There were no significant differences in the reduction in inflammatory markers between the 2 treatments.

Disease activity, assessed by DAS-28, fell by a similar degree following both ezetimibe and simvastatin, and there was no significant difference between the treatments. When looking at the individual components of DAS-28, the only parameters that reached statistical significance were ESR and tender joints count. Neither mean arterial pressure nor AIx was significantly affected by either drug. However, aortic PWV was significantly reduced (Fig. 2) and FMD increased (Fig. 3) following both drugs, and there was no change in the GTN response. (The p values in Figures 2 and 3 are Bonferroni adjusted.) There were no significant differences in the hemodynamic effects of the 2 treatments.

Custom hypothesis testing for the effect of treatment order showed that the order in which the drugs were received did not affect any of the outcomes ($p < 0.05$) and there was no carryover effect of the previous treatment after the 6-week washout period (unpaired *t* test between 2 baselines: $p < 0.05$). In pooled data of both treatments, a reduction in total, LDL, and oxLDL cholesterol were found to correlate with the improvement of FMD ($r = -0.5$, -0.5, and -0.6, respectively; all $p < 0.05$). We did not find a correlation between cholesterol reduction and improvement of inflammatory markers or disease activity. There was a significant correlation between baseline CRP and change in CRP ($r = -0.8$; $p < 0.001$) and between baseline ESR

Table 1 Baseline Demographics, Biochemical, and Hemodynamic Characteristics of Subjects With Rheumatoid Arthritis (n = 20)

Age, yrs	58 ± 12
Disease duration, yrs	13 ± 10
Female, n (%)	16 (80%)
BMI, kg/m^2	26.4 ± 4.6
TC, mmol/l	5.3 ± 0.9
TG, mmol/l	1.3 ± 0.5
LDL cholesterol, mmol/l	3.1 ± 0.9
HDL cholesterol, mmol/l	1.6 ± 0.6
Glucose, mmol/l	4.8 ± 0.5
Rheumatoid factor positive, n (%)	14 (70%)
CRP, mg/l	8.97 ± 0.56*
ESR, mm/h	16 ± 1*
DAS-28	4.65 ± 1.44
SBP, mm Hg	137 ± 17
DBP, mm Hg	84 ± 11
MAP, mm Hg	102 ± 12
Heart rate, beats/min	75 ± 12
AP, mm Hg	13 ± 7
AIx, %	29 ± 10
aPWV, m/s	9.43 ± 2.42

Values represent mean ± standard deviation, unless otherwise indicated. *Data are geometric mean ± standard deviation, calculated from log-transformed distribution.

AIx = augmentation index; AP = augmentation pressure; aPWV = aortic pulse wave velocity; CRP = C-reactive protein; DAS= Disease Activity Score; DBP = diastolic blood pressure; ESR = erythrocyte sedimentation rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MAP = mean arterial pressure; SBP = systolic blood pressure; TC = total cholesterol; TG = triglycerides.

Table 2 Effect of Treatment on Lipids, Inflammation, Disease Activity, and Hemodynamics After 6 Weeks of Treatment

					Significance	
	Baseline	Ezetimibe	Baseline	Simvastatin	Overall	Between Drugs
TC, mmol/l	5.3 ± 1.0	4.7 ± 1.0§	5.4 ± 0.9	4.1 ± 0.7§	<0.001	<0.001
TG, mmol/l	1.5 ± 0.7	1.3 ± 0.7†	1.4 ± 0.5	1.3 ± 0.8	0.02	0.8
LDL, mmol/l	3.08 ± 0.92	2.53 ± 0.94§	3.18 ± 0.78	1.95 ± 0.56§	<0.001	0.001
HDL, mmol/l	1.59 ± 0.55	1.65 ± 0.57	1.62 ± 0.51	1.65 ± 0.43	0.4	0.7
TC/HDL ratio	3.67 ± 1.44	3.11 ± 1.51‡	3.63 ± 1.44	2.64 ± 0.78§	<0.001	0.1
oxLDL, U/l	61.4 ± 16.6	55.7 ± 18.4†	63.0 ± 17.5	43.3 ± 14.1§	<0.001	0.003
ESR, mm/h*	18.2 ± 15.6	12.9 ± 9.6†	18.6 ± 12.4	13.8 ± 8.9†	0.006	0.9
CRP, mg/l*	14.2 ± 14.7	8.8 ± 9.4‡	15.3 ± 15.2	10.3 ± 10.4§	0.002	0.9
DAS-28	4.41 ± 1.20	3.86 ± 1.57†	4.65 ± 1.35	3.98 ± 1.31‡	0.002	0.7
TJC	8.45 ± 6.28	7.30 ± 7.37	10.20 ± 8.33	7.40 ± 7.27†	0.03	0.3
SJC	5.50 ± 4.44	4.30 ± 6.44	5.45 ± 5.37	4.65 ± 6.00	0.4	0.8
VAS	33.85 ± 20.61	33.80 ± 21.32	38.15 ± 22.55	36.85 ± 21.10	0.8	0.8
SBP, mm Hg	135 ± 19	134 ± 20	136 ± 19	135 ± 18	0.4	0.8
DBP, mm Hg	79 ± 9	80 ± 9	82 ± 9	81 ± 10	0.9	0.1
MAP, mm Hg	97 ± 10	99 ± 11	100 ± 11	98 ± 11	0.6	0.1
AP, mm Hg	15 ± 9	14 ± 7	15 ± 8	15 ± 8	0.3	0.3
AIX, %	27 ± 11	26 ± 10	28 ± 11	27 ± 10	0.3	0.9
aPWV, m/s	9.54 ± 2.52	8.85 ± 2.12†	9.59 ± 2.30	8.88 ± 2.06§	0.001	0.9
Baseline diameter, mm	4.13 ± 0.93	4.18 ± 0.74	4.04 ± 0.88	4.05 ± 0.93	0.7	0.7
FMD, %	3.65 ± 2.60	5.01 ± 3.46†	3.88 ± 2.48	6.40 ± 3.78‡	0.001	0.1
GTN, %	8.39 ± 3.37	8.49 ± 3.40	8.03 ± 2.70	8.10 ± 3.41	0.9	0.9

Values represent mean ± standard deviation. Significance was determined using 2-way repeated measures analysis of variance (ANOVA). *Log-transformed values were used for the analyses of the skewed variables. †$p < 0.025$; ‡$p < 0.01$; §$p < 0.001$; custom hypothesis testing of within-subject contrasts was performed for the comparison of ezetimibe versus simvastatin (final column): the effect of individual treatments was determined in post hoc tests with Bonferroni adjustment for 2 comparisons, when overall significance in ANOVA was $p < 0.05$.

FMD = flow-mediated dilation; GTN = glyceryl trinitrate; oxLDL = oxidized low-density lipoprotein; SJC = swollen joints count; TJC = tender joints count; VAS = visual analog score; other abbreviations as in Table 1.

and change in aortic PWV, with greatest reductions in PWV occurring in subjects with the highest baseline ESR (Fig. 4).

The baseline aortic PWV was significantly increased in patients with RA compared with control subjects (9.42 ± 2.42 m/s vs. 7.69 ± 1.18 m/s, respectively; p = 0.005) and FMD was reduced (3.70 ± 2.32% vs. 6.74 ± 3.78%, respectively; p = 0.01). Following ezetimibe and simvastatin, FMD improved to a level similar to the control subjects (p = 0.2 and p = 0.8, respectively), but aortic PWV remained elevated (p = 0.02). There was no significant change in AIx following either drug.

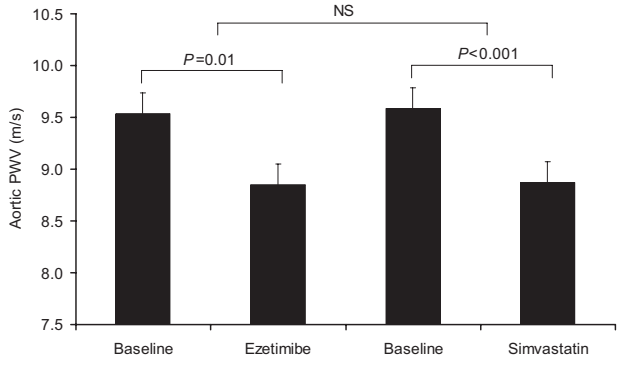

Figure 2 Effect of 10 mg Ezetimibe and 20 mg Simvastatin on Aortic PWV

Measurements were taken after 6 weeks of each treatment. **Bars** show mean and standard error of mean (n = 20). The omnibus significance for the trend was p = 0.001. The significance was determined by 2-way repeated measures analysis of variance with post hoc testing with Bonferroni adjustment. PWV = pulse wave velocity.

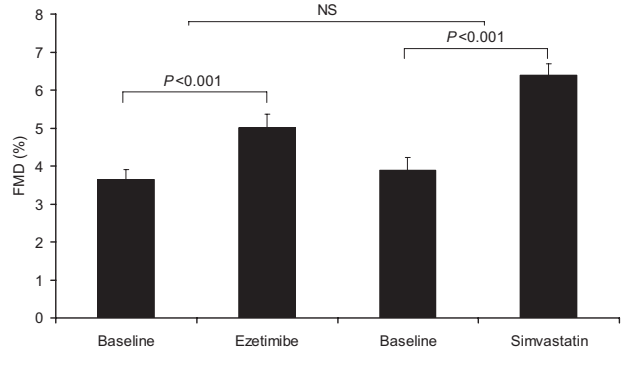

Figure 3 Effect of 10 mg Ezetimibe and 20 mg Simvastatin on FMD

Measurements were taken after 6 weeks of each treatment. **Bars** show mean and standard error of mean (n = 20). The omnibus significance for the trend was p < 0.001. The significance was determined by 2-way repeated measures analysis of variance with post hoc testing with Bonferroni adjustment. FMD = flow-mediated dilation.

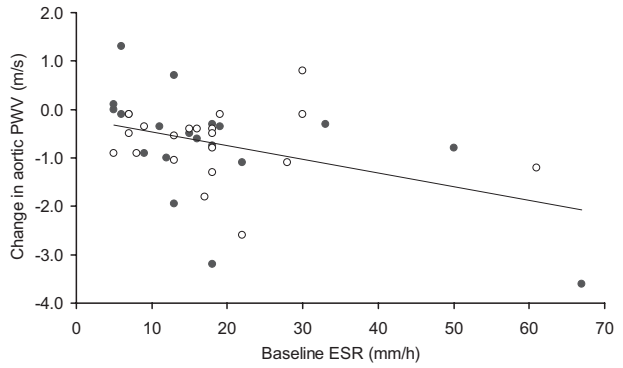

Figure 4 **Relationship Between Baseline ESR and Change in Aortic PWV Following Ezetimibe and Simvastatin**

The change in aortic pulse wave velocity (PWV) following drug treatment was significantly associated with the baseline erythrocyte sedimentation rate (ESR); r = −0.416; p = 0.008. **Solid circles** = ezetimibe; **open circles** = simvastatin.

Discussion

In the present study, we demonstrated that short-term therapy with both ezetimibe and simvastatin significantly reduces cholesterol, inflammatory markers, and disease activity in patients with active RA. Interestingly, despite a significantly greater reduction in cholesterol following treatment with simvastatin, the anti-inflammatory effects of the two drugs were similar. Moreover, we have shown for the first time, in a single cohort of RA patients, that both ezetimibe and simvastatin reduce aortic PWV and improve endothelial function.

The recent TARA study showed that statins reduce inflammation and disease activity in patients with RA (15). However, it is unclear to what extent this effect is due to cholesterol reduction or true pleiotropism, because there was no control cholesterol-lowering therapy. It is also unclear whether treatment is associated with any improvement in surrogate measures of cardiovascular risk. Therefore, in the present study we assessed the effect of simvastatin as well as of the nonstatin cholesterol-lowering agent ezetimibe on inflammation, disease activity, and surrogate measures of cardiovascular risk, such as endothelial function and aortic PWV, in a cohort of subjects with active RA.

Anti-inflammatory effects. Treatment with ezetimibe and simvastatin produced similar reductions in inflammatory markers and disease activity. The improvement of DAS-28 was mostly due to the reduction of ESR, but there was also a significant reduction in tender joints count. Although, the reduction in inflammatory markers correlated with the degree of baseline inflammation, there was no correlation between the extent of cholesterol reduction and fall in CRP or DAS-28, which was also the case in the TARA study (15). This suggests either a potential "threshold effect" or that the reduction in cholesterol induced by ezetimibe and simvastatin was not sufficiently different, or indeed large, to unmask such a relationship.

Treatment with statins has been consistently associated with a decrease in inflammatory markers (14,19,20) in a variety of patient groups, including subjects with RA (15). However, previous data suggest that ezetimibe only reduces CRP when given in combination with other agents (19,20). Indeed, the present study is the first to show an anti-inflammatory effect of ezetimibe per se. This may relate to the relatively high level of systemic inflammation associated with RA patients compared with subjects with pure hypercholesterolemia or cardiovascular disease included in earlier studies (19,20). The present data are also the first to show that ezetimibe produces a clinical reduction of inflammation and disease activity in subjects with RA. It is unlikely that any such effects of ezetimibe are pleiotropic, because it is not absorbed into the circulation but acts locally by inhibiting cholesterol absorption from the small intestine. Taken together, these observations suggest that cholesterol reduction per se has anti-inflammatory effects in patients with RA. This notion is supported by previous observations that fibrates (21) and systemic Acyl-CoA cholesterol acyltransferase (ACAT) inhibition (22) reduce inflammatory markers in other patient groups.

Reduction of oxLDL. The anti-inflammatory effects seen in the present study may be mediated by the reduction in oxLDL. Indeed, oxLDL is known to increase the expression of proinflammatory genes, leading to monocyte adhesion to arterial endothelial cells (23,24). Moreover, recent evidence from cultured human coronary artery endothelial cells indicates that oxLDL, through its receptor LOX-1, activates an inflammatory reaction by up-regulating CD40 and CD40L signaling pathways, and CD40 antibody reduces oxLDL-induced tumor necrosis factor (TNF)-alpha production (25). This may be especially important in RA, which is associated with elevated TNF-alpha signaling and increased oxidative stress driven by high inflammation (26). This could initiate a vicious circle, where inflammation-driven oxidation of LDL leads to further increases in inflammation, worsening of disease activity, and yet further oxidation, ultimately leading to endothelial dysfunction and cardiovascular disease. The present results support this theory by demonstrating that despite having LDL levels similar to control subjects, RA patients had higher oxLDL levels and worse endothelial function at baseline.

Reduction of arterial stiffness. We confirmed our original observations of increased aortic PWV in RA and for the first time demonstrated a significant reduction in aortic PWV with antihyperlipidemic drugs in patients with RA. The reduction in aortic PWV correlated with the baseline ESR, with greatest reductions in PWV occurring in subjects with the highest ESR. This finding is in line with other studies, where greater clinical benefits of statins have been demonstrated in patients with high levels of inflammation (27,28). These data also extend our previous observations that anti–TNF-alpha therapy improves endothelial function and reduces aortic stiffness in RA (9) and suggest that even

modest reductions in systemic inflammation may lead to beneficial effects on surrogates of cardiovascular risk.

We did not find any significant change in AIx following either drug. This suggests that despite reduction in wave speed, there was no reduction in the impact of wave reflection. This could be due to a fall in inflammation and subsequent peripheral vasoconstriction, which would lead to increased impedance mismatch at the point of reflection, and therefore the net effect on AIx would remain unchanged. Our data contradict those of Efrati et al. (29), who demonstrated that 40 mg simvastatin, but not 80 mg simvastatin, 10 mg ezetimibe, or combination of 40 mg simvastatin and 10 mg ezetimibe, reduces AIx. However, those data were based on small parallel groups (n = 10).

Improvement of endothelial function. Statins improve endothelial function in patients with hypercholesterolemia (30), heart failure (31), coronary artery disease (CAD) (32), and RA (33). Most authors have ascribed this to the pleiotropic effect of statins, but, with the exception of 3 studies (29,31,32), investigators have not controlled for the cholesterol reduction. In the present study, we demonstrated for the first time that both ezetimibe and simvastatin significantly improve endothelial function in RA to a level similar to healthy control subjects. Although the improvement in FMD following simvastatin appeared greater than that following ezetimibe, it did not reach statistical significance. However, we cannot rule out the possibility that we would have reached a statistical significance if a larger number of patients were studied. Importantly, there was no change in the GTN response or baseline diameter of the brachial artery between visits, suggesting that smooth muscle susceptibility to nitric oxide (NO) was not altered. The reduction of total, LDL, and oxLDL cholesterol significantly correlated with the improvement of FMD, suggesting that cholesterol, and especially oxLDL, reduction per se improves endothelial function. Our findings contradict those of Landmesser et al. (31) and Fichtlscherer et al. (32), who showed that only simvastatin and not ezetimibe improved endothelial function. However, those studies were performed in patients with heart failure and CAD, not RA, which may explain the different findings. Moreover, although anti-TNF therapy has been associated with improved cardiovascular outcome in patients with RA (34), it worsens outcome in heart failure (35). Furthermore, the study by Landmesser et al. (31) included only 20 patients in a parallel group design, whereas ours employed the same number of subjects in a crossover design, providing greater power.

Possible mechanisms behind the improvement of endothelial function. Support for the notion that cholesterol reduction per se may improve endothelial vasomotor function comes from in vivo and in vitro observations. A number of nonstatin therapies, such as a single LDL apheresis (36), fibrates (21,37), and systemic ACAT inhibition (22) have already been linked with improved endothelial function in hypercholesterolemic patients. Moreover, in vitro LDL itself inhibits endothelium-dependent vasodilatation (38). The composition of lipid rafts within the plasma membrane is also dependent on the circulating lipid profile. Indeed, the expression of scaffolding protein caveolin is increased in hypercholesterolemia (39). Caveolin functions as an inhibitor of endothelial nitric oxide synthase (eNOS) by blocking access of eNOS to its cofactor and substrate, thus reducing NO production (40) and possibly leading to endothelial dysfunction. Therefore, lipid reduction may lead to decreased expression of caveolin and to restoration of normal transport of substrate L-arginine for eNOS (41).

Study limitations. This study was powered to detect a change in CRP and DAS-28 with statin therapy, and our power calculations were based on the reductions seen in the TARA study, but it was not powered to detect a difference of <50% between the 2 treatments. The crossover design of the study is an obvious strength, but also a weakness owing to a potential carryover effect. Nevertheless, we did not find a carryover effect after a 6-week washout period, and the order of the drugs did not affect the outcome. We did not have a combined simvastatin and ezetimibe treatment period and therefore we cannot answer whether this would have added to the effect we observed.

Furthermore, as with all chronic diseases, the disease activity can fluctuate markedly during the course of a study, especially if the duration is long. Therefore, our treatment period was relatively short.

Conclusions

We have shown that both ezetimibe and simvastatin reduce inflammatory markers ESR and CRP as well as disease activity to a similar degree in patients with RA. We have also shown, for the first time in a randomized double-blind study, that endothelial function and concomitantly arterial stiffness were improved by both drugs. These results suggest that the reduction of cholesterol per se ameliorates aortic stiffness and endothelial dysfunction. Our data also suggest that cholesterol-reducing therapies may be beneficial for RA patients, because they are well tolerated, improve clinical outcome. and reduce surrogates of cardiovascular risk. Future studies are needed to establish whether a reduction of arterial stiffness and improvement of endothelial function with antihyperlipidemic agents translates to an improvement in cardiovascular outcome in patients with RA.

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REFERENCES

1. Wolfe F, Mitchell DM, Sibley JT, et al. The mortality of rheumatoid arthritis. Arthritis Rheum 1994;37:481–94.
2. del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum 2001;44:2737–45.
3. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation 1999;99:2434–9.
4. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948–54.
5. Hansel S, Lassig G, Pistrosch F, Passauer J. Endothelial dysfunction in young patients with long-term rheumatoid arthritis and low disease activity. Atherosclerosis 2003;170:177–80.
6. Bergholm R, Leirisalo-Repo M, Vehkavaara S, Makimattila S, Taskinen MR, Yki-Jarvinen H. Impaired responsiveness to NO in newly diagnosed patients with rheumatoid arthritis. Arterioscler Thromb Vasc Biol 2002;22:1637–41.
7. Turesson C, Jacobsson L, Ryden AA, Sturfelt G, Wollmer P, Lanne T. Increased stiffness of the abdominal aorta in women with rheumatoid arthritis. Rheumatology (Oxford) 2005;44:896–901.
8. Roman MJ, Devereux RB, Schwartz JE, et al. Arterial stiffness in chronic inflammatory diseases. Hypertension 2005;46:1–6.
9. Maki-Petaja KM, Hall FC, Booth AD, et al. Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by antitumor necrosis factor-alpha therapy. Circulation 2006;114:1185–92.
10. Hurlimann D, Forster A, Noll G, et al. Anti–tumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. Circulation 2002;106:2184–7.
11. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383–9.
12. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:7–22.
13. Halcox JP, Deanfield JE. Beyond the laboratory: clinical implications for statin pleiotropy. Circulation 2004;109:II42–II48.
14. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E, Cholesterol and Recurrent Events (CARE) Investigators. Long-term effects of pravastatin on plasma concentration of C-reactive protein. Circulation 1999;100:230–5.
15. McCarey DW, McInnes IB, Madhok R, et al. Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet 2004;363:2015–21.
16. Wilkinson IB, Fuchs SA, Jansen IM, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 1998;16:2079–84.
17. Mullen MJ, Thorne SA, Deanfield JE, Jones CJ. Non-invasive assessment of endothelial function. Heart 1997;77:297–8.
18. Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.
19. Sager PT, Melani L, Lipka L, et al. Effect of coadministration of ezetimibe and simvastatin on high-sensitivity C-reactive protein. Am J Cardiol 2003;92:1414–8.
20. Ballantyne CM, Houri J, Notarbartolo A, et al. Effect of ezetimibe coadministered with atorvastatin in 628 patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. Circulation 2003;107:2409–15.
21. Kon KK, Yeal AJ, Hwan HS, et al. Effects of fenofibrate on lipoproteins, vasomotor function, and serological markers of inflammation, plaque stabilization, and hemostasis. Atherosclerosis 2004;174:379–83.
22. Kharbanda RK, Wallace S, Walton B, Donald A, Cross JM, Deanfield J. Systemic Acyl-CoA:cholesterol acyltransferase inhibition reduces inflammation and improves vascular function in hypercholesterolemia. Circulation 2005;111:804–7.
23. Hansson GK. Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol 2001;21:1876–90.
24. Li D, Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. Circulation 2000;101:2889–95.
25. Li D, Liu L, Chen H, Sawamura T, Mehta JL. LOX-1, an oxidized LDL endothelial receptor, induces CD40/CD40L signaling in human coronary artery endothelial cells. Arterioscler Thromb Vasc Biol 2003;23:816–21.
26. Remans PH, van Oosterhout M, Smeets TJ, et al. Intracellular free radical production in synovial T lymphocytes from patients with rheumatoid arthritis. Arthritis Rheum 2005;52:2003–9.
27. Ridker PM, Rifai N, Pfeffer MA, et al., Cholesterol and Recurrent Events (CARE) Investigators. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Circulation 1998;98:839–44.
28. Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001;344:1959–65.
29. Efrati S, Averbukh M, Dishy V, Faygenzo M, Friedensohn L, Golik A. The effect of simvastatin, ezetimibe and their combination on the lipid profile, arterial stiffness and inflammatory markers. Eur J Clin Pharmacol 2007;63:113–21.
30. O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation 1997;95:1126–31.
31. Landmesser U, Bahlmann F, Mueller M, et al. Simvastatin versus ezetimibe: pleiotropic and lipid-lowering effects on endothelial function in humans. Circulation 2005;111:2356–63.
32. Fichtlscherer S, Schmidt-Lucke C, Bojunga S, et al. Differential effects of short-term lipid lowering with ezetimibe and statins on endothelial function in patients with CAD: clinical evidence for "pleiotropic" functions of statin therapy. Eur Heart J 2006;27:1182–90.
33. Hermann F, Forster A, Chenevard R, et al. Simvastatin improves endothelial function in patients with rheumatoid arthritis. J Am Coll Cardiol 2005;45:461–4.
34. Jacobsson LT, Turesson C, Gulfe A, et al. Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. J Rheumatol 2005;32:1213–8.
35. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the AntiTNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003;107:3133–40.
36. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL apheresis improves endothelium-dependent vasodilatation in hypercholesterolemic humans. Circulation 1997;95:76–82.
37. Evans M, Anderson RA, Graham J, et al. Ciprofibrate therapy improves endothelial function and reduces postprandial lipemia and oxidative stress in type 2 diabetes mellitus. Circulation 2000;101:1773–9.
38. Andrews HE, Bruckdorfer KR, Dunn RC, Jacobs M. Low-density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. Nature 1987;327:237–9.
39. Feron O, Dessy C, Moniotte S, Desager JP, Balligand JL. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. J Clin Invest 1999;103:897–905.
40. Ju H, Zou R, Venema VJ, Venema RC. Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. J Biol Chem 1997;272:18522–5.
41. Mason RP, Walter MF, Jacob RF. Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. Circulation 2004;109:II34–41.